Standard Extraction Method
PRIOR ART

Dry lipids under nitrogen
⇓

Sonicate in buffer to form micelles
Add reaction mixture
⇓

Stop Reaction
⇓

Extract lipids in chloroform/methanol
to induce two phases
⇓

Spin down
⇓

Remove upper (water) phase
⇓

To lower phase add
artificial upper phase
⇓

Spin down
⇓

Remove lower (lipid) phase
into fresh tubes
⇓

Count or run TLC

Present Invention

Spot liquids on the membrane directly
from chloroform/methanol solution
⇓

Add reaction mixture
(enzyme + ^{32}P-ATP)
⇓

Stop reaction
⇓

Wash membranes
⇓

Phosphoimage analysis or
radioactivity counting

FIG. 1

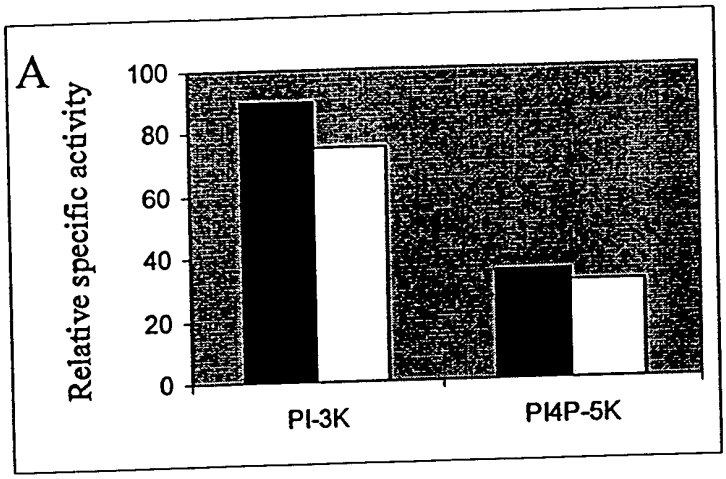

A
Relative specific activity
100
80
60
40
20
0
PI-3K
PI4P-5K

FIG. 2A

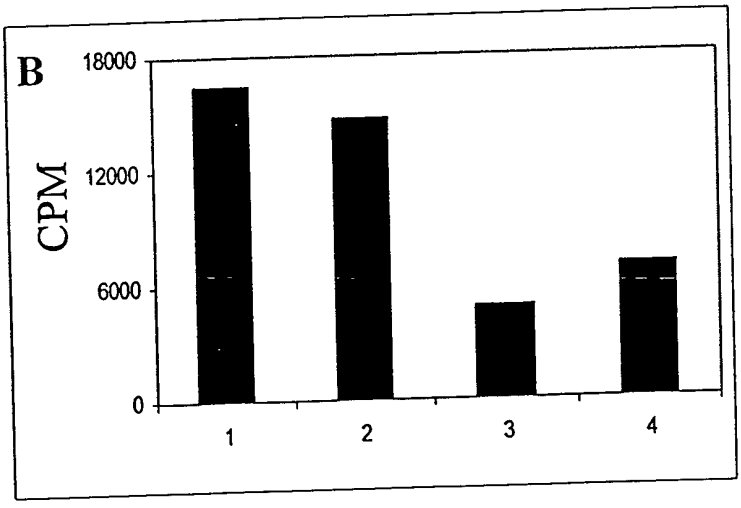

B
CPM
18000
12000
6000
0
1
2
3
4

FIG. 2B

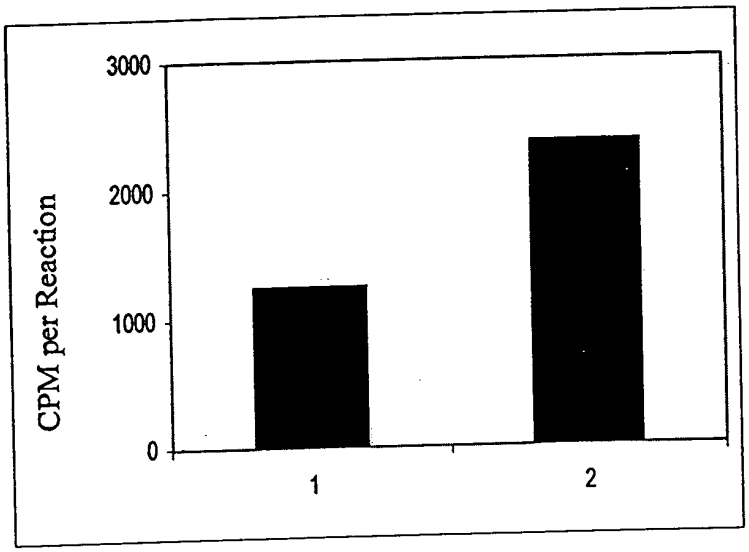


FIG. 3

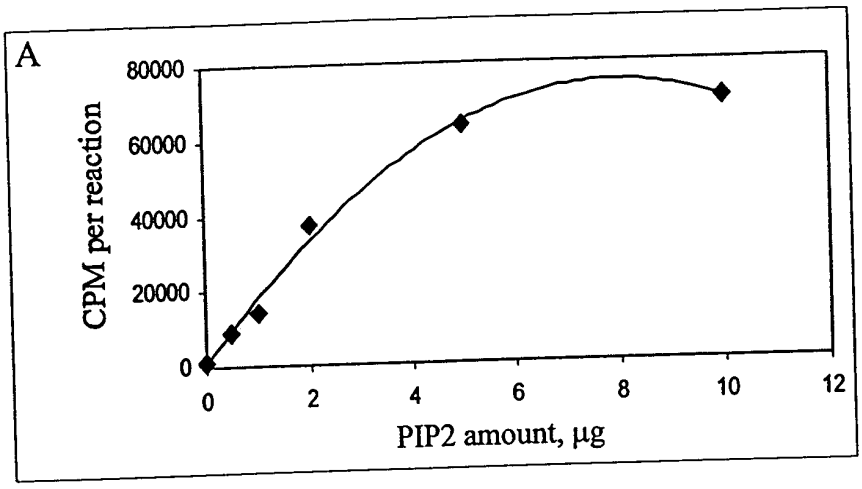

A
80000
60000
40000
20000
0
CPM per reaction
0
2
4
6
8
10
12
PIP2 amount, µg

FIG. 4A

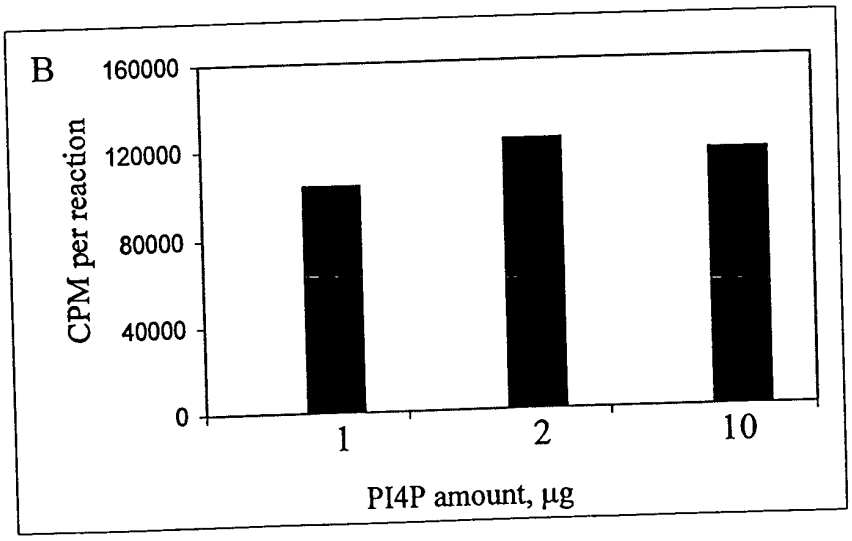

B
160000
120000
80000
40000
0
CPM per reaction
1
2
10
PI4P amount, µg

FIG. 4B

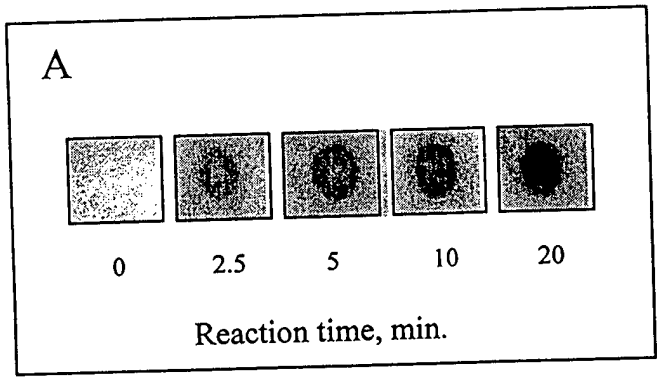


FIG. 5A

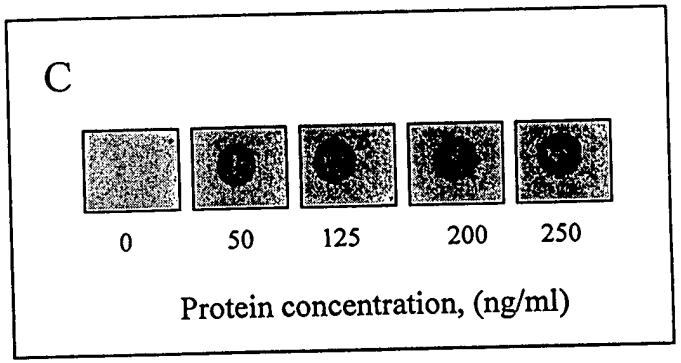


FIG. 5C

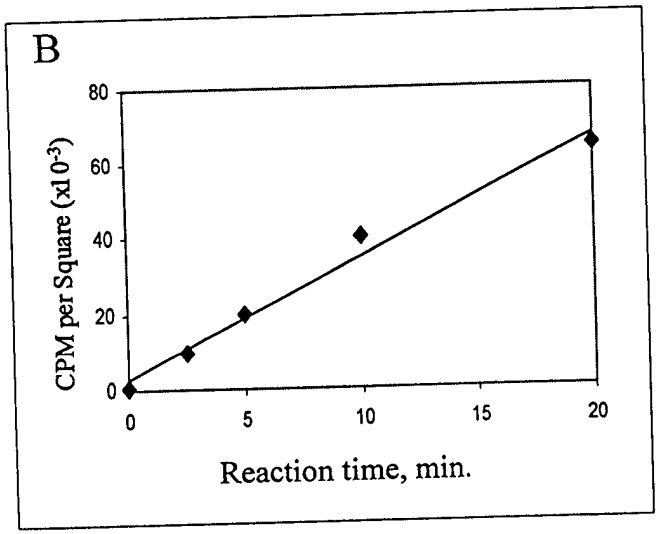


FIG. 5B

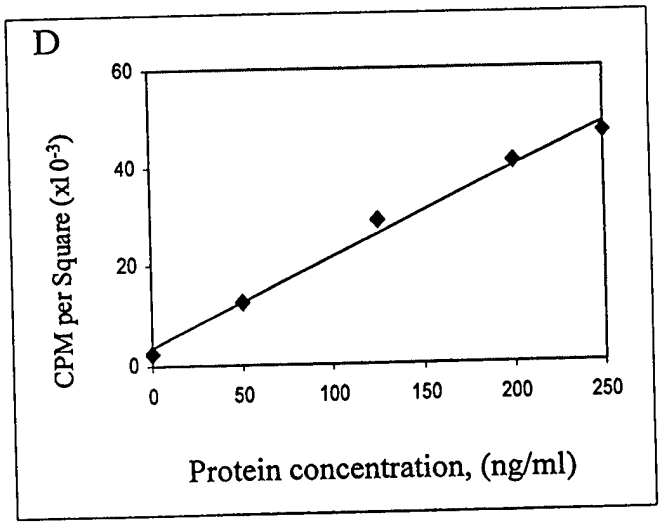


FIG. 5D

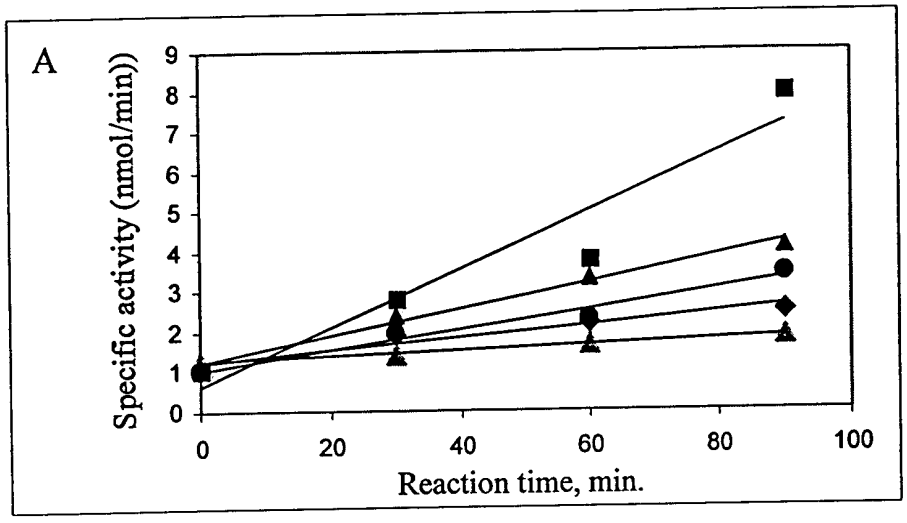

A
Specific activity (nmol/min))
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
Reaction time, min.

FIG. 6A

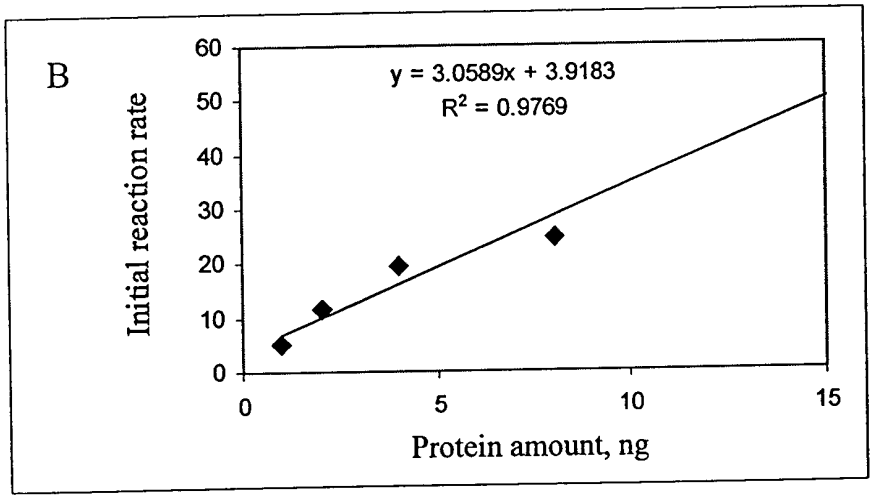

B
Initial reaction rate
60
50
40
30
20
10
0
y = 3.0589x + 3.9183
R2 = 0.9769
0
5
10
15
Protein amount, ng

FIG. 6B

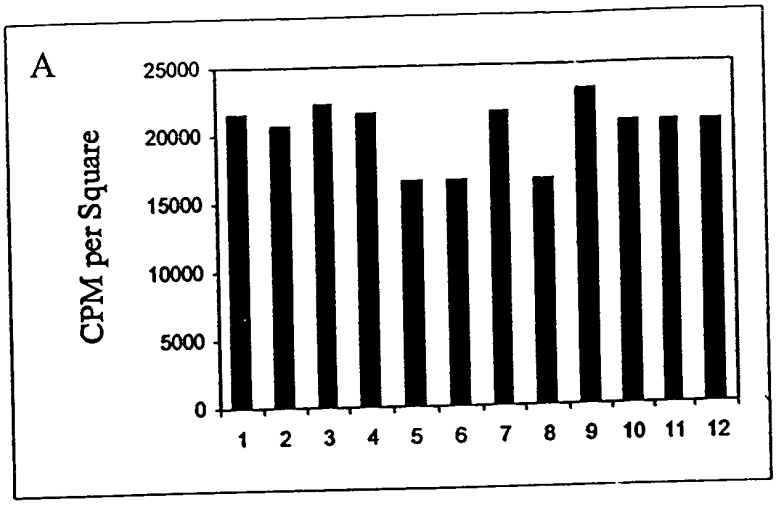


FIG. 7A

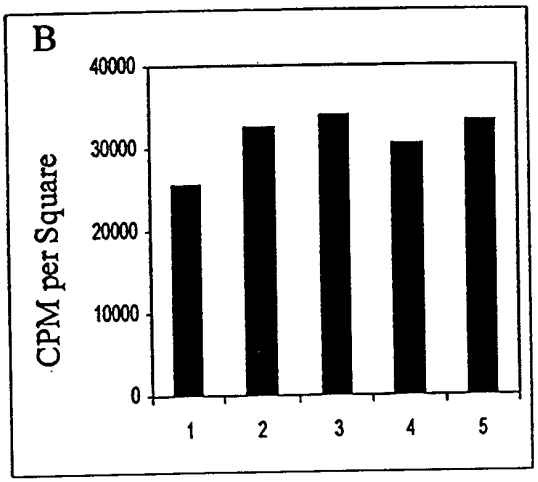


FIG. 7B

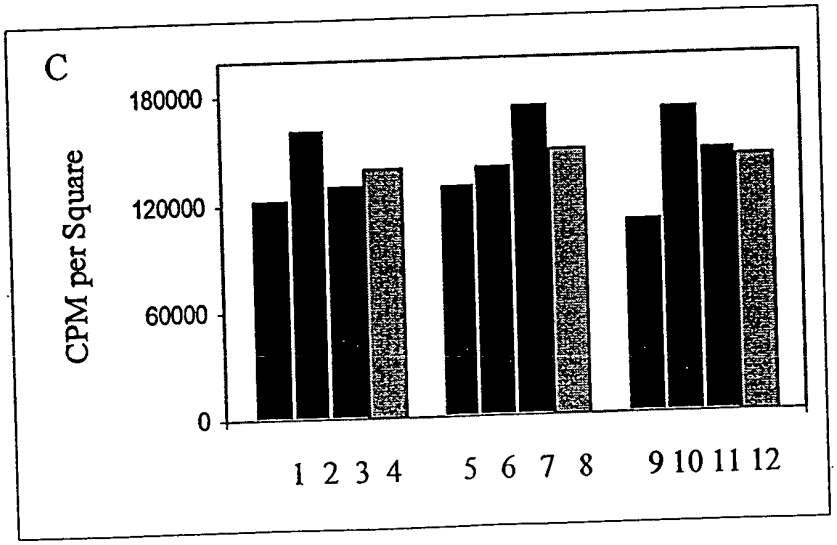


FIG. 7C

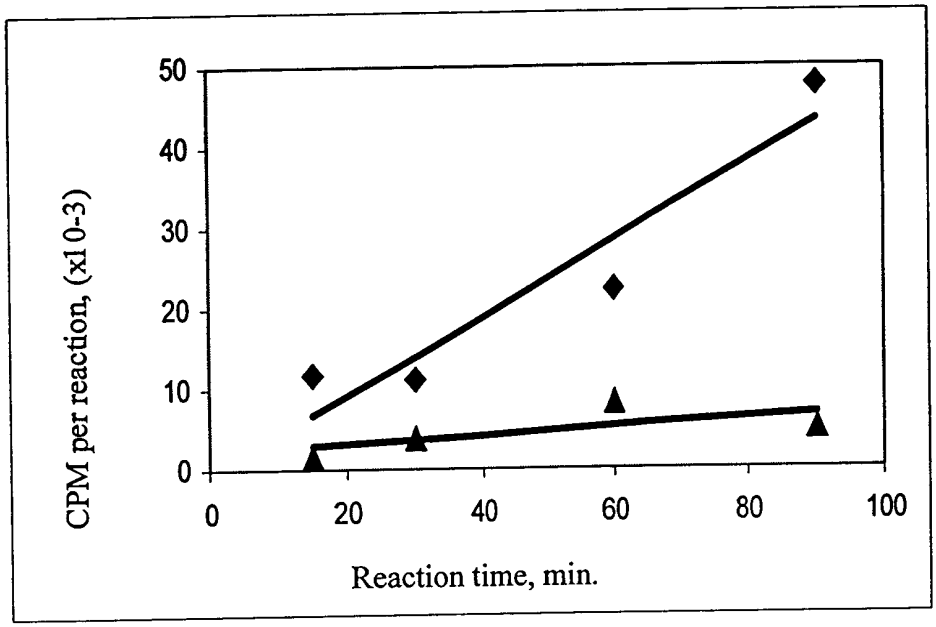

CPM per reaction, (x10-3)
50
40
30
20
10
0
0
20
40
60
80
100
Reaction time, min.

FIG. 8

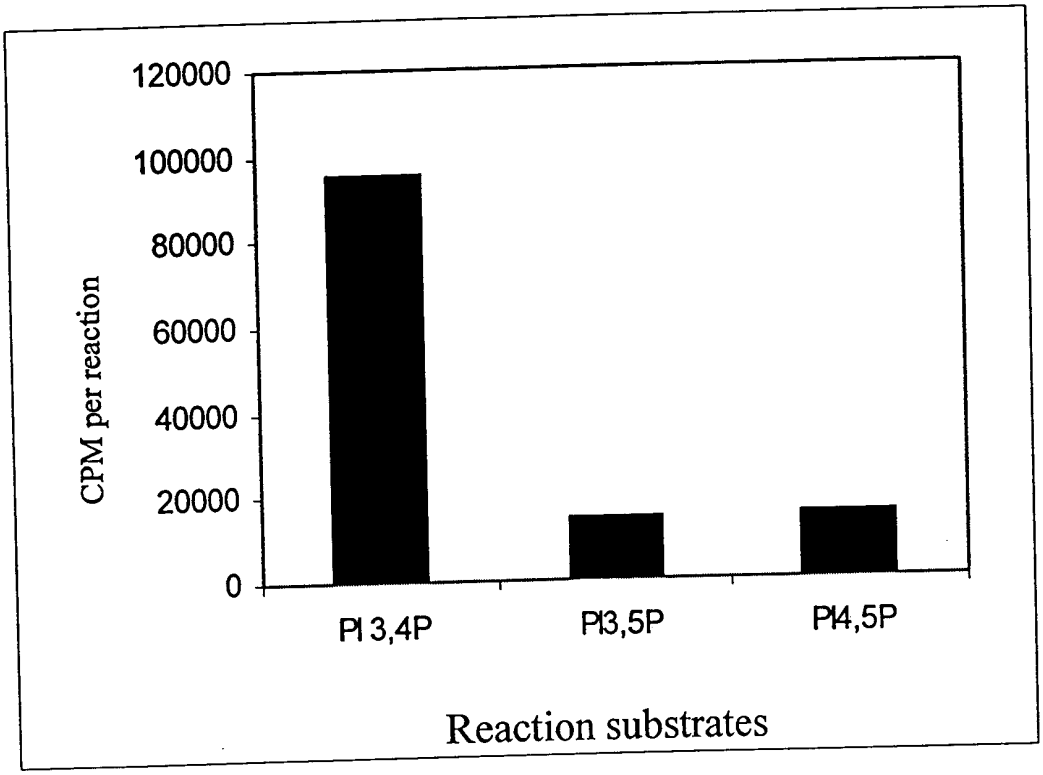

120000
100000
80000
60000
40000
20000
0
CPM per reaction
PI 3,4P
PI3,5P
PI4,5P
Reaction substrates

FIG. 9

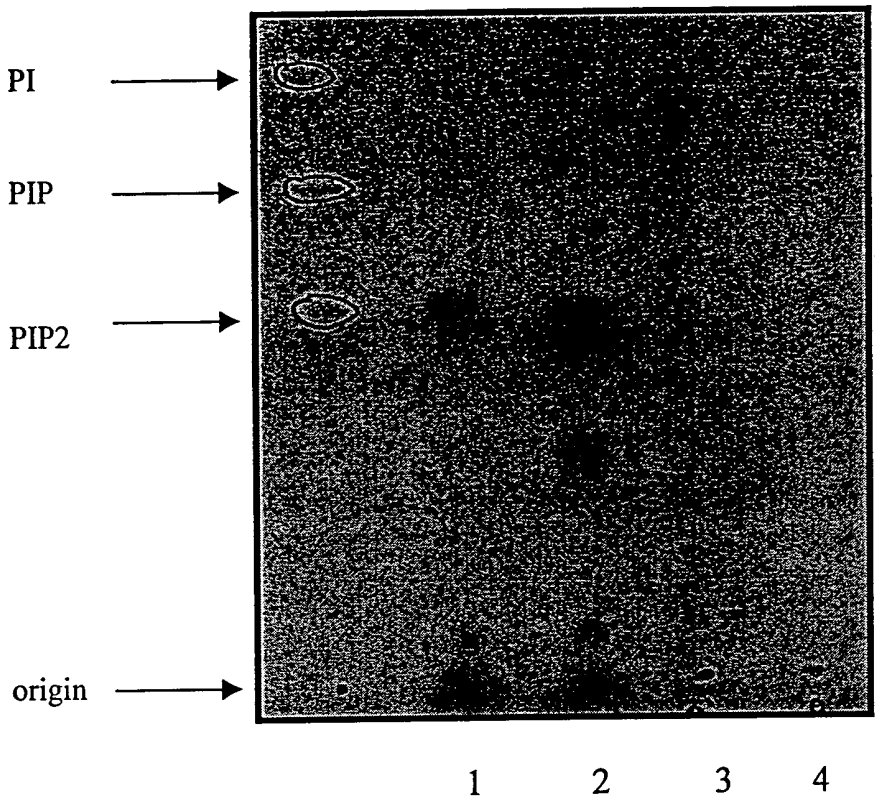


FIG. 10

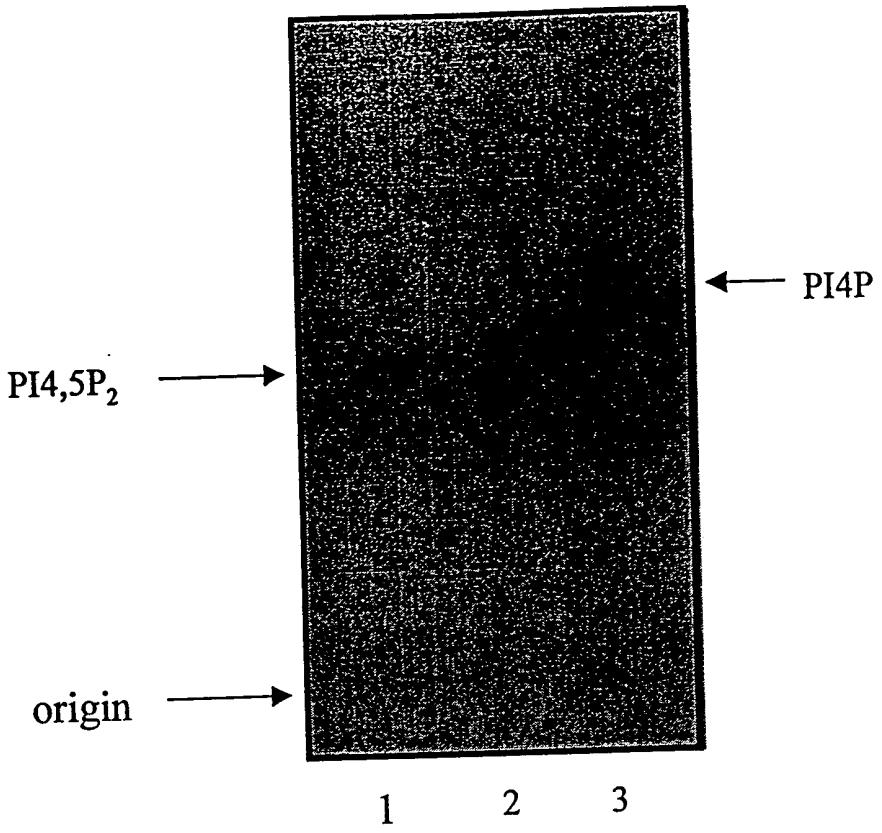


FIG. 11A

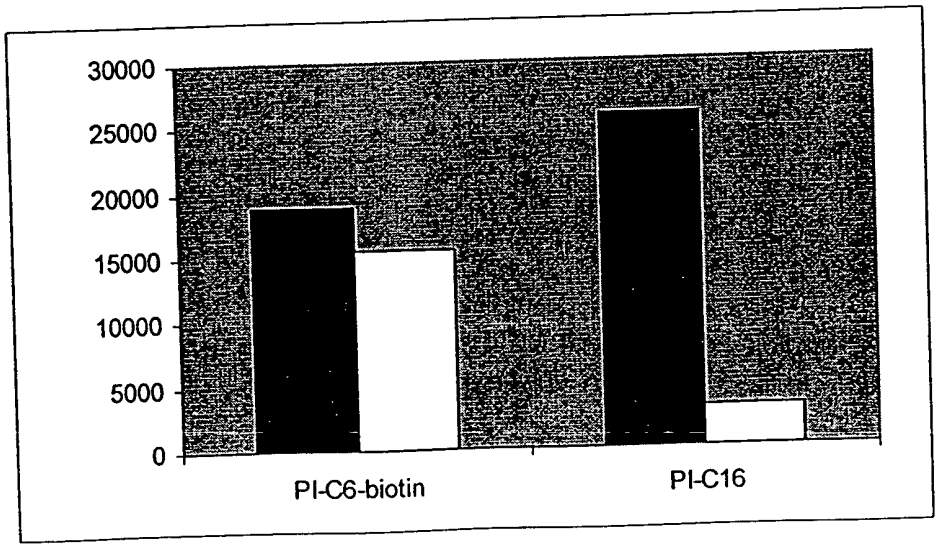


FIG. 11B